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A natural peptide with NMDA inhibitory activity reduces tonic pain in the formalin model

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THE aim of this study was to assess whether a natural peptide, histogranin, isolated from chromaffin cells and possessing NMDA receptor inhibitory activity, could reduce tonic pain. Rats received intrathecal injections of the stable analog [Ser[1]]histogranin (SHG), prior to induction of the formalin response. SHG markedly suppressed the second tonic phase of the formalin response compared with saline vehicle. A U-shaped dose–response curve was obtained. SHG had no effect on phase 1 acute pain responses. These findings indicate that SHG acts in a similar fashion as other, non-peptide, NMDA antagonists in suppressing tonic, but not acute pain. The presence of the natural peptide in chromaffin cells may contribute to the analgesic effects of adrenal medullary implants.

Key words: Adrenal medulla; Analgesia; Chromaffin cells; Chronic pain; Neuropeptides; NMDA antagonists; Spinal cord

Introduction

Recent theories regarding the mechanisms of chronic pain suggest that excessive activation of NMDA receptors in the spinal cord initiates a cascade of events leading to long-term neuroplastic changes and persistent hyperexcitability.[1–4] In support of this, several groups have demonstrated that treatment with NMDA antagonists can reduce abnormal hyperalgesia resulting from peripheral nerve injury or inflammation.[5–7] The biphasic nociceptive response in the formalin model has been used to distinguish pharmacological mechanisms in the development of persistent pain. Following a transient acute phase, a more prolonged tonic phase develops which may involve central sensitization similar to that found in persistent pain syndromes. The tonic phase is thought to be initiated by activation of NMDA receptors as it is suppressed by NMDA antagonists.[8–10]

Work in our laboratory has demonstrated that transplantation of adrenal medullary chromaffin cells into the spinal subarachnoid space can reduce persistent pain behaviors in several models.[11–14] A potential mechanism for this pain reduction is via blockade of the NMDA-initiated cascade in the spinal cord, as adrenal medullary transplants can attenuate hyperalgesia and allodynia induced by direct intrathecal injection of NMDA.[12] Recent findings have demonstrated that these transplants suppress the tonic phase of the formalin test, and this is not reversed by opiate or α-adrenergic antagonists.[13] In addition to opioid peptides and catecholamines, chromaffin cells produce a variety of other neuroactive peptides and neurotrophic factors. A novel peptide recently isolated from bovine adrenal medulla, histogranin, is found in high concentrations in chromaffin granules.[15] This pentadecapeptide inhibits ligand binding to NMDA receptors, and both it and its stable analogue [Ser[1]]histogranin (SHG) block NMDA-induced convulsions in a dose-dependent fashion.[15,16] The goal of the present study was to determine whether SHG may act as a natural NMDA inhibitor to attenuate the development of persistent pain. Preliminary findings from this study have been reported previously.[17]

Materials and Methods

SHG was custom synthesized by Research and Diagnostic Antibodies (Berkeley, CA). All procedures involving animals were reviewed and approved by the institutional animal care committee. Male Sprague–Dawley rats (300–350 g, Sasco, WI; $n = 20$) were anesthetized with pentobarbital (40 mg/kg, i.p., supplemented as necessary) and implanted with intrathecal catheters as described elsewhere.[12] Catheters were threaded through the spinal subarachnoid space from a slit in the atlanto-occipital membrane to the level of the lumbar enlargement. Following a 2-week recovery period, animals were acclimatized individually in open Plexiglas chambers

for unobstructed observation during formalin testing. Fifteen minutes prior to formalin injection, animals received an intrathecal injection of either SHG (0.5, 1.0, 2.0 or 4.0 μg/15 μl) or saline vehicle, followed by a 10 μl flush with saline. To induce the formalin response, 50 μl of 5% formalin was injected into the plantar surface of the right hindpaw with a 30 G needle. The incidence of spontaneous flinching of the injected paw was counted during the first minute following injection, and for 1 min periods every 5 min thereafter for 60 min, similar to methods described elsewhere.[10,13] Statistical comparisons between treatment groups were made using ANOVA (repeated measures for time course) and the Newman-Keuls test for multiple *post hoc* comparisons (SigmaStat, Jandel Scientific).

Results

Figure 1A shows the time course and magnitude of the formalin response in animals injected intrathecally with either SHG (1.0 μg) or saline vehicle. In animals receiving saline, a typical biphasic formalin response was observed, with rapid flinching in the first minute following formalin injection, followed by a quiescent period at ~5–15 min, and then a second, prolonged flinching phase which peaked in severity at 45 min and began to subside thereafter. Intrathecal injection of SHG markedly attenuated formalin-induced flinching (overall $F(1,12) = 89.0$; $p < 0.001$). This attenuation was limited to the tonic phase ($p < 0.05$ compared with saline at all time points after 10 min). Thus, SHG had no effect on the acute phase of the formalin response ($p > 0.05$ compared with saline). Similarly, none of the other doses of SHG produced significant attenuation of the first phase flinching response ($p > 0.05$ for all doses of SHG compared with saline in phase 1).

In order to compare the dose-effectiveness of SHG in suppressing the second phase of the formalin response, mean flinching responses observed between 30 and 55 min following formalin injection were calculated for each animal. Group means at each SHG dose are plotted in Fig. 1B. Results revealed a U-shaped dose-response curve (overall $F(4,19) = 48.4$, $p < 0.001$). Peak attenuation of flinching behavior was obtained with the 1.0 μg dose of SHG ($p < 0.05$ compared with all other doses). The lower dose (0.5 μg) produced a smaller but statistically significant suppression of flinching ($p < 0.05$ compared with saline). Interestingly, higher doses (2.0 and 4.0 μg) of SHG were less effective, although some suppression of flinching behavior was still observed ($p < 0.05$ compared with saline).

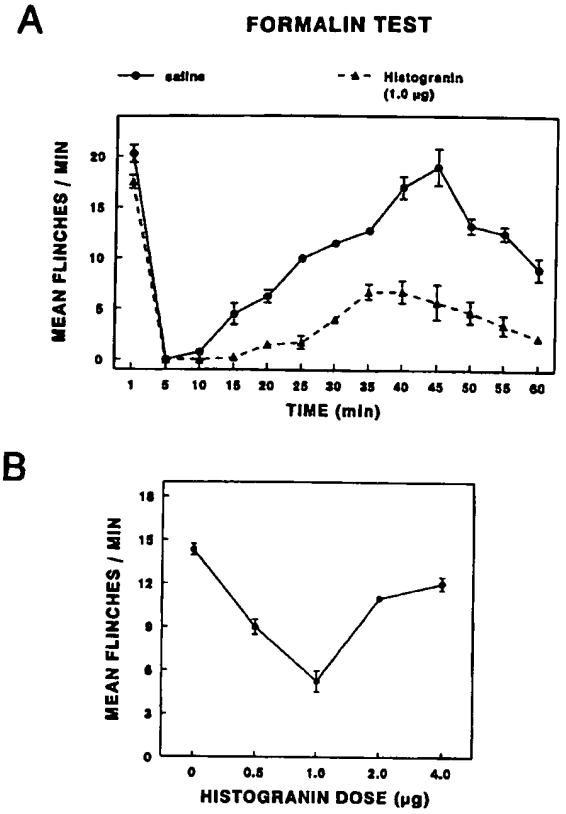

FIG. 1. Effect of intrathecal [Ser¹]histogranin (SHG) on formalin-induced flinching responses. (**A**) Time course of the biphasic flinching response following intrathecal injection of SHG. The ordinate is number of flinches/min in the first minute following formalin injection and every 5 min thereafter up to 60 min. Points represent the mean ± s.e.m. for animals injected with either SHG (1.0 μg; triangles) or saline vehicle (circles). (**B**) Dose–response relationship between several doses of SHG and mean (± s.e.m.) flinching behavior in the second phase of the formalin response (30–55 min post-formalin).

Discussion

The results of this study demonstrate that a novel natural peptide can reduce tonic pain in the formalin model. The pentadecapeptide, histogranin, was originally isolated from the adrenal medulla and is associated with the chromaffin granule fraction, suggesting a neuropeptide function.[15] Using an antibody against the synthetic stable analog SHG, this peptide has also been localized in the pituitary, brain and blood plasma.[15] Charactization of this peptide in other laboratories has suggested that it possesses potential NMDA antagonist-like activity.[15,16,18] In rat brain membrane preparations, [^{125}I]SHG binding is specific ($K_d = 25$ nM) and regionally distributed with higher levels in brain regions known to possess high NMDA receptor densities.[18] Both histogranin and SHG displace the binding of [^{3}H]CGP39653, a specific ligand of the NMDA receptor.[15,16] Further,

histogranin or SHG injected i.c.v. can protect against NMDA-induced convulsions, but not convulsions induced by AMPA, kainate or bicuculline.[15,16] The binding site for histogranin may be unique, since [^{125}I]SHG binding is not displaced by ligands of the binding domains of the NMDA receptor[18] or by specific ligands for PCP, opioid, dopaminergic, adrenergic or cholinergic receptors.[19] Polyamine site-specific agonists and antagonists are effective in reducing [^{125}I]SHG binding in a non-competitive fashion, but the lack of reciprocity suggests to these authors that the histogranin receptor is distinct from the polyamine binding domain and that interaction between these two sites is allosteric.[18,19]

Current theories regarding chronic pain mechanisms suggest that abnormal persistent pain processes involve initial activation of spinal NMDA receptors.[1–4] As a model for this, the biphasic formalin response is thought to be mediated by pharmacologically distinct mechanisms, the second phase predictive of chronic pain and sensitive to NMDA antagonists[8–10] The results of the present study demonstrating suppression of the second, but not the first phase by SHG is consistent with the hypothesis that histogranin may act as an endogenous antagonist of the NMDA receptor. However, while all doses utilized suppressed flinching behavior to some extent, an intermediate dose was most effective, resulting in a U-shaped dose–response curve. This suggests the possibility that histogranin may be a weak or partial agonist, thus displacing other more potent ligands from the NMDA receptor at lower doses, prior to weakly activating the receptor itself at higher doses. Alternatively, in light of binding studies described above, SHG may indirectly inhibit NMDA receptor mediated events at sites distinct from the NMDA binding domains.

Our laboratory has been focused on the use of neural transplantation for alleviation of pain. Adrenal medullary chromaffin cells were originally selected as donors for this purpose, as they secrete catecholamines and opioid peptides, agents which can reduce nociception when administered intrathecally.[20] However, recent findings in our laboratory have suggested that adrenal medullary transplants may attenuate persistent pain via an alternative mechanism, as suppression of the tonic phase of the formalin test by these transplants is not reversed by opiate or α-adrenergic antagonists.[13] This may involve interference with the NMDA-initiated cascade, since adrenal medullary transplants can reduce hyperalgesia and allodynia induced by direct intrathecal injection of NMDA.[12] The results of the present study suggest that a potential candidate for this is histogranin, since it is also present in high quantities in the adrenal medulla.

Conclusion

Results of the present study demonstrate that a naturally derived mammalian peptide may provide NMDA inhibitory activity useful in the suppression of persistent pain. In addition, the actions of histogranin may contribute to the beneficial effects of adrenal medullary transplants in the alleviation of chronic pain.

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General Summary

Recent theories regarding abnormal persistent pain suggest that it involves activation of spinal NMDA receptors. Our laboratory has demonstrated that adrenal medullary transplants can reduce symptoms of chronic pain, possibly by interfering with the NMDA-initiated cascade. A natural peptide, histogranin, originally identified in the adrenal medulla, possesses NMDA inhibitory activity. To assess whether this peptide could suppress pain, rats were injected intrathecally with the stable analog [Ser^1]histogranin (SHG) or saline vehicle prior to induction of the formalin pain response. SHG markedly suppressed flinching in the tonic pain phase, but not in the acute phase, consistent with other NMDA antagonists. A U-shaped dose–response curve was obtained, suggesting the possibility of partial agonist activity. Results of the present study demonstrate that a naturally derived mammalian peptide may provide NMDA inhibitory activity useful in suppression of persistent pain. In addition, actions of histogranin may contribute to the beneficial effects of adrenal medullary transplants in alleviating chronic pain.